SEQUENCE LISTING

```
<110> Ding, Jeak Ling
Ho, Bow
National University of Singapore
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<120> Sushi Peptide Multimer

<130> 040184-000400US

<140> US 10/563,551

<141> 2006-01-04

<150> CA 2,432,972

<151> 2003-07-04

<150> WO PCT/SG04/00194

<151> 2004-07-02

<160> 11

<170> PatentIn Ver. 2.1

<210> 1

<211> 34

<212> PRT

<213> Artificial Sequence

<220×

<223> Description of Artificial Sequence:synthetic
 34-mer Sushi-3 peptide (S3 peptide), residues
 268-301 of Factor C, Sushi3 domain, LPS-binding
 motif

<400> 1

His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln 1 5 10 15

Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe 20 25 30

Leu Met

<210> 2

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:synthetic 34-mer Sushi-3delta peptide (S3delta peptide)

<400> 2

His Ala Glu His Lys Val Lys Ile Lys Val Lys Gln Lys Tyr Gly Gln 1 5 10 15

Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe 20 25 30

Leu Met

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<210> 3
<211> 38
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: LPS-binding
      motif S3 PCR amplification forward primer
<400> 3
tcgaagacgg ccccaggatc cccatgctga acacaagg
                                                                   38
<210> 4
<211> 35
<212> DNA
<213> Artificial Sequence
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<223> Description of Artificial Sequence: LPS-binding
      motif S3 PCR amplification reverse primer
<400> 4
tagaagaccc gggggtccat caaagaaagt agtta
                                                                    35
<210> 5
<211> 3448
<212> DNA
<213> Carcinoscorpius rotundicauda
<220>
<223> Factor C cDNA
<220>
<221> CDS
<222> (18)..(3077)
<223> Factor C
<400> 5
gtgaaggtaa cttaagt atg gtc tta gcg tcg ttt ttg gtg tct ggt tta
                                                                    50
                   Met Val Leu Ala Ser Phe Leu Val Ser Gly Leu
gtt cta ggg cta cta gcc caa aaa atg cgc cca gtt cag tcc aaa gga
                                                                    98
Val Leu Gly Leu Leu Ala Gln Lys Met Arg Pro Val Gln Ser Lys Gly
gta gat cta ggc ttg tgt gat gaa acg agg ttc gag tgt aag tgt ggc
                                                                    146
Val Asp Leu Gly Leu Cys Asp Glu Thr Arg Phe Glu Cys Lys Cys Gly
gat cca ggc tat gtg ttc aac att cca gtg aaa caa tgt aca tac ttt
                                                                    194
Asp Pro Gly Tyr Val Phe Asn Ile Pro Val Lys Gln Cys Thr Tyr Phe
tat cga tgg agg ccg tat tgt aaa cca tgt gat gac ctg gag gct aag
                                                                    242
Tyr Arg Trp Arg Pro Tyr Cys Lys Pro Cys Asp Asp Leu Glu Ala Lys
60
                     65
                                          70
```

| | t tgt le Cys | | | | | | | | | | | | | | 290 | |
|------------------|--------------------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|------------|------------|------------|-------------------|------------|-----|-----|
| | gt tgt er Cys | | | | | | | | | | | | | | 338 | |
| | aa tgt lu Cys 110 | | | | | | | | | | | | | | 386 | |
| Ala Cy | gt gca ys Ala 25 | | | | | | | | | | | | | | 434 | |
| | gt tgt ly Cys | | | | | | | | | | | | | | 482 | |
| | ca cca ro Pro | _ | | | | | | _ | | _ | _ | | | | 530 | |
| | ag ctt ys Leu | _ | | _ | _ | _ | | _ | _ | | | | | _ | 578 | |
| | gt aac er Asn 190 | | | | | | | | | | | | | | 626 | 3 |
| Ser Pr | ca gaa co Glu 05 . | | | | | | | | | | | | | | 674 | • • |
| | ct act la Thr | | | | | | | _ | | | | _ | | | 722 | |
| caa ga Gln Gl | aa aca lu Thr | tta Leu | acc Thr 240 | tgt Cys | cag Gln | ggt Gly | aat Asn | ggt Gly 245 | cag Gln | tgg Trp | aat Asn | gga Gly | cag Gln 250 | ata Ile | 770 | |
| | aa tgt ln Cys | | | | | | | | | | | | | | 818 | |
| _ | ct gaa la Glu 270 | | _ | _ | | | | | _ | | | | | _ | 866 | |
| | ct caa co Gln 35 | | | | | | | | | | | | | | 914 | |
| | g ggt et Gly | | | | | | | | | | | | | | 962 | |

| | | | | | tgt Cys | | | | | | | | 1010 |
|-------------------|---|---|---|---|-------------------|---|-------|---|---|---|---|---|------|
| | | | | | gac Asp | | | | | | | | 1058 |
| | | | | | ggc Gly | | | | | | | | 1106 |
| | | | | | gaa Glu | | | | | | | | 1154 |
| | | | | | aac Asn 385 | | | | | | | | 1202 |
| | | | - | _ | ttt Phe | _ | _ | - | _ | | | _ | 1250 |
| | - | _ | _ | | ctt Leu | _ | _ | | _ | - | _ | _ | 1298 |
| | | | | | aaa Lys | | | | | | | _ | 1346 |
| Asp | | | | | tac Tyr | | | | | | | | 1394 |
| gct Ala 460 | | | | | | | | | | | | | 1442 |
| aaa Lys | | | | | aat Asn | | | | | | | | 1490 |
| tta Leu | | | | | | | | | | | | | 1538 |
| | | | | | atg Met | | | | | | | | 1586 |
| cta Leu | | | | | tct Ser | | | | | | | | 1634 |
| | | | | | gat Asp 545 | | | | | | | | 1682 |

•

| | | | | | | | | tgc Cys | | | | | | | 1730 | | | |
|------------|---|---|---|---|---|---|---|-------------------|---|---|---|---|---|---|----------|----|--|--|
| | | | | | | | | gga Gly 580 | | | | | | | 1778 | | | |
| | | | | | | | | Gly 999 | | | | | | | 1826 | | | |
| | | | | | | | | tac Tyr | | | | | | | 1874 | | | |
| | | | | | | | | agt Ser | _ | | | | _ | _ | 1922 | | | |
| | | _ | | _ | | | | cct Pro | _ | | | | | | 1970 | | | |
| ٠. | _ | | | | _ | | | aac Asn 660 | _ | | _ | _ | _ | | 2018 | | | |
| <i>:</i> . | | | | | | | | ccc Pro | | | | | | | 2066 | ٠. | | |
| Ĵ | | | | | | | | tca Ser | | | | | - | | 2114 | | | |
| | | | | | | | | ggt Gly | | | | | | | 2162 | | | |
| | _ | | _ | _ | | _ | _ | tac Tyr | | _ | | | | | 2210 | | | |
| | | | | | | | | aac Asn 740 | | | | | | | 2258 | | | |
| | | | | | | | | gac Asp | | | | | | | 2306 | | | |
| | | | | | | | | ggt Gly | | | | | | | 2354 | | | |
| | | | | | | | | aat Asn | | | | | | | 2402 | | | |

| gga to Gly Se | | | | | | | | | | | | | | | 2450 |
|-------------------------|----------------|-------------------|------------|------------|-------------------|------------|-------------------|------------|------------|-------------------|------------|-------------------|------------|------------|------|
| acc ta Thr Ty | | | | | | | | | | | | | | | 2498 |
| tat ct Tyr Le | | | | | | | | | | | | _ | | | 2546 |
| caa gt Gln Va 84 | l Arg | | | | | | | | | | | | | | 2594 |
| ggc aa Gly As 860 | | | | | | | | | | | | | | | 2642 |
| act tt Thr Le | _ | | _ | _ | | | | _ | _ | | | _ | | | 2690 |
| aca ag Thr Ar | | | | | | | | | | | | | | | 2738 |
| ggt tt Gly Le | | | | | | | | | | | | | | | 2786 |
| cta cc Leu Pr 92 | o Val | gtt Val | gca Ala | gcc Ala | agc Ser 930 | acc Thr | tgt Cys | gaa Glu | gag Glu | 999 Gly 935 | tac Tyr | aag Lys | gaa Glu | gca Ala | 2834 |
| gac tt Asp Le 940 | | | | | | | | | | | | | | | 2882 |
| aag gg Lys Gl | | | | | | | | | | | | | | | 2930 |
| ttt gc Phe Al | t gat a Asp | gat Asp 975 | tcc Ser | cgt Arg | acc Thr | gaa Glu | agg Arg 980 | cgg Arg | tgg Trp | gtc Val | ttg Leu | gaa Glu 985 | Gly aaa | att Ile | 2978 |
| gtc ag Val Se | | | | | | | | | | Ala | | | | | 3026 |
| ggc tt Gly Ph 100 | e Thr | | _ | Asn | _ | | _ | | Trp | | | _ | | | 3074 |
| tga aa | ctgat | cta a | aatat | ttta | aa go | catgo | gttat | aaa | acgto | ttg | tttc | cctat | ta | | 3127 |
| 1020 | | | | | | | | | | | | | | | |

ttgctttact agtttaaccc ataagaaggt taactgggta aggcacaagg atcattgttt 3187

ctgtttgttt ttacaaatgg ttattttagt cagtgaatga gaatagtatc cattgaagac 3247
tgttaccttt tattctacct ttttatatta ctatgtaagt atttgggata tcttctacac 3307
atgaaaattc tgtcatttta ccataaattt ggtttctggt gtgtgctaag tccaccagta 3367
gagaacgatg taattttcac tagcacatga aataaatata gaacaaatct attataaact 3427
accttaaaaa aaaaaaaaaa a

<210> 6 <211> 1019 <212> PRT <213> Carcinoscorpius rotundicauda <220> <223> Factor C <400> 6 Met Val Leu Ala Ser Phe Leu Val Ser Gly Leu Val Leu Gly Leu Leu 10 Ala Gln Lys Met Arg Pro Val Gln Ser Lys Gly Val Asp Leu Gly Leu 2.0 25 Cys Asp Glu Thr Arg Phe Glu Cys Lys Cys Gly Asp Pro Gly Tyr Val 40 Phe Asn Ile Pro Val Lys Gln Cys Thr Tyr Phe Tyr Arg Trp Arg Pro 55 Tyr Cys Lys Pro Cys Asp Asp Leu Glu Ala Lys Asp Ile Cys Pro Lys 70 Tyr Lys Arg Cys Gln Glu Cys Lys Ala Gly Leu Asp Ser Cys Val Thr 85 90 Cys Pro Pro Asn Lys Tyr Gly Thr Trp Cys Ser Gly Glu Cys Gln Cys 105 Lys Asn Gly Gly Ile Cys Asp Gln Arg Thr Gly Ala Cys Ala Cys Arg 125 Asp Arg Tyr Glu Gly Val His Cys Glu Ile Leu Lys Gly Cys Pro Leu Leu Pro Ser Asp Ser Gln Val Gln Glu Val Arg Asn Pro Pro Asp Asn 155 Pro Gln Thr Ile Asp Tyr Ser Cys Ser Pro Gly Phe Lys Leu Lys Gly Met Ala Arg Ile Ser Cys Leu Pro Asn Gly Gln Trp Ser Asn Phe Pro 185 Pro Lys Cys Ile Arg Glu Cys Ala Met Val Ser Ser Pro Glu His Gly 200 Lys Val Asn Ala Leu Ser Gly Asp Met Ile Glu Gly Ala Thr Leu Arg 215 220 Phe Ser Cys Asp Ser Pro Tyr Tyr Leu Ile Gly Gln Glu Thr Leu Thr 230 235 Cys Gln Gly Asn Gly Gln Trp Asn Gly Gln Ile Pro Gln Cys Lys Asn 245 250 Leu Val Phe Cys Pro Asp Leu Asp Pro Val Asn His Ala Glu His Lys 265 Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr 280 Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Gly Phe Asp 295 300 Thr Leu Lys Cys Asn Pro Asp Gly Ser Trp Ser Gly Ser Gln Pro Ser

315

310

Cys Val Lys Val Ala Asp Arg Glu Val Asp Cys Asp Ser Lys Ala Val Asp Phe Leu Asp Asp Val Gly Glu Pro Val Arg Ile His Cys Pro Ala Gly Cys Ser Leu Thr Ala Gly Thr Val Trp Gly Thr Ala Ile Tyr His Glu Leu Ser Ser Val Cys Arg Ala Ala Ile His Ala Gly Lys Leu Pro Asn Ser Gly Gly Ala Val His Val Val Asn Asn Gly Pro Tyr Ser Asp Phe Leu Gly Ser Asp Leu Asn Gly Ile Lys Ser Glu Glu Leu Lys Ser Leu Ala Arg Ser Phe Arg Phe Asp Tyr Val Arg Ser Ser Thr Ala Gly Lys Ser Gly Cys Pro Asp Gly Trp Phe Glu Val Asp Glu Asn Cys Val Tyr Val Thr Ser Lys Gln Arg Ala Trp Glu Arg Ala Gln Gly Val Cys Thr Asn Met Ala Ala Arg Leu Ala Val Leu Asp Lys Asp Val Ile Pro Asn Ser Leu Thr Glu Thr Leu Arg Gly Lys Gly Leu Thr Thr Trp Ile Gly Leu His Arg Leu Asp Ala Glu Lys Pro Phe Ile Trp Glu Leu Met Asp Arg Ser Asn Val Val Leu Asn Asp Asn Leu Thr Phe Trp Ala Ser Gly Glu Pro Gly Asn Glu Thr Asn Cys Val Tyr Met Asp Ile Gln Asp Gln Leu Gln Ser Val Trp Lys Thr Lys Ser Cys Phe Gln Pro Ser Ser Phe Ala Cys Met Met Asp Leu Ser Asp Arg Asn Lys Ala Lys Cys Asp Asp Pro Gly Ser Leu Glu Asn Gly His Ala Thr Leu His Gly Gln Ser Ile Asp Gly Phe Tyr Ala Gly Ser Ser Ile Arg Tyr Ser Cys Glu Val Leu His Tyr Leu Ser Gly Thr Glu Thr Val Thr Cys Thr Thr Asn Gly Thr Trp Ser Ala Pro Lys Pro Arg Cys Ile Lys Val Ile Thr Cys Gln Asn Pro Pro Val Pro Ser Tyr Gly Ser Val Glu Ile Lys Pro Pro Ser Arg Thr Asn Ser Ile Ser Arg Val Gly Ser Pro Phe Leu Arg Leu Pro Arg Leu Pro Leu Pro Leu Ala Arg Ala Ala Lys Pro Pro Pro Lys Pro Arg Ser Ser Gln Pro Ser Thr Val Asp Leu Ala Ser Lys Val Lys Leu Pro Glu Gly His Tyr Arg Val Gly Ser Arg Ala Ile Tyr Thr Cys Glu Ser Arg Tyr Tyr Glu Leu Leu Gly Ser Gln Gly Arg Arg Cys Asp Ser Asn Gly Asn Trp Ser Gly Arg Pro Ala Ser Cys Ile Pro Val Cys Gly Arg Ser Asp Ser Pro Arg Ser Pro Phe Ile Trp Asn Gly Asn Ser Thr Glu Ile Gly Gln Trp Pro Trp Gln Ala Gly Ile Ser Arg Trp Leu Ala Asp His Asn Met Trp Phe Leu Gln Cys Gly Gly Ser Leu Leu Asn

```
Glu Lys Trp Ile Val Thr Ala Ala His Cys Val Thr Tyr Ser Ala Thr
               805
                                   810
Ala Glu Ile Ile Asp Pro Asn Gln Phe Lys Met Tyr Leu Gly Lys Tyr
           820
                               825
Tyr Arg Asp Asp Ser Arg Asp Asp Tyr Val Gln Val Arg Glu Ala
                          840
Leu Glu Ile His Val Asn Pro Asn Tyr Asp Pro Gly Asn Leu Asn Phe
                       855
                                           860
Asp Ile Ala Leu Ile Gln Leu Lys Thr Pro Val Thr Leu Thr Thr Arg
                   870
                                      875
Val Gln Pro Ile Cys Leu Pro Thr Asp Ile Thr Thr Arg Glu His Leu
               885
                                   890
Lys Glu Gly Thr Leu Ala Val Val Thr Gly Trp Gly Leu Asn Glu Asn
           900
                               905
Asn Thr Tyr Ser Glu Thr Ile Gln Gln Ala Val Leu Pro Val Val Ala
       915
                           920
                                              925
Ala Ser Thr Cys Glu Glu Gly Tyr Lys Glu Ala Asp Leu Pro Leu Thr
                       935
                                          940
Val Thr Glu Asn Met Phe Cys Ala Gly Tyr Lys Lys Gly Arg Tyr Asp
                   950
                                       955
Ala Cys Ser Gly Asp Ser Gly Gly Pro Leu Val Phe Ala Asp Asp Ser
               965
                                   970
Arg Thr Glu Arg Arg Trp Val Leu Glu Gly Ile Val Ser Trp Gly Ser
           980
                               985
Pro Ser Gly Cys Gly Lys Ala Asn Gln Tyr Gly Gly Phe Thr Lys Val
       995 1000
Asn Val Phe Leu Ser Trp Ile Arg Gln Phe Ile
   1010
                      1015
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```
<210> 7
```

<220>

<223> Description of Artificial Sequence:recombinant S3
 monomer (rS3-lmer) peptide, with additional Pro
 and Asp at ends from acid cleavage of rS3-4mer DP
 linker

<400> 7

Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr Gly
1 5 10 15

Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr 20 25 30

Phe Leu Met Asp 35

<210> 8

<211> 468

<212> DNA

<213> Artificial Sequence

<211> 36

<212> PRT

<213> Artificial Sequence

<220> <223> Description of Artificial Sequence:recombinant S3 tetramer (rS3-4mer) tandem repeat peptide with acid cleavable DP linker between S3 units <220> <221> CDS <222> (1)..(468) <223> rS3-4mer <400> 8 ccc cag gat ccc cat gct gaa cac aag gtt aaa att ggt gtg gaa caa 48 Pro Gln Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln aaa tat ggt cag ttt cct caa ggc act gaa gtg acc tat acg tgt tcg 96 Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser 25 ggt aac tac ttc ttg atg gac ccc cag gat ccc cat gct gaa cac aag 144 Gly Asn Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys 35 gtt aaa att ggt gtg gaa caa aaa tat ggt cag ttt cct caa ggc act 192 Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr 50 55 gaa gtg acc tat acg tgt tcg ggt aac tac ttc ttg atg gac ccc cag 240 Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp Pro Gln 70 gat ccc cat gct gaa cac aag gtt aaa att ggt gtg gaa caa aaa tat Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr ggt cag ttt cct caa ggc act gaa gtg acc tat acg tgt tcg ggt aac 336 Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn 100 tac ttc ttg atg gac ccc cag gat ccc cat gct gaa cac aag gtt aaa 384 Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys Val Lys 115 120 att ggt gtg gaa caa aaa tat ggt cag ttt cct caa ggc act gaa gtg 432 Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val 130 135 acc tat acg tgt tcg ggt aac tac ttc ttg atg gac 468 Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp 145 150 <210> 9 <211> 156 <212> PRT <213> Artificial Sequence <220> <223> Description of Artificial Sequence:recombinant S3 tetramer (rS3-4mer) tandem repeat peptide with

acid cleavable DP linkerbetween S3 units

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<400> 9
Pro Gln Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln
Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser
                                 25
Gly Asn Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys
                             40
Val Lys Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr
Glu Val Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp Pro Gln
Asp Pro His Ala Glu His Lys Val Lys Ile Gly Val Glu Gln Lys Tyr
Gly Gln Phe Pro Gln Gly Thr Glu Val Thr Tyr Thr Cys Ser Gly Asn
                                105
Tyr Phe Leu Met Asp Pro Gln Asp Pro His Ala Glu His Lys Val Lys
        115
Ile Gly Val Glu Gln Lys Tyr Gly Gln Phe Pro Gln Gly Thr Glu Val
                        135
Thr Tyr Thr Cys Ser Gly Asn Tyr Phe Leu Met Asp
                    150
<210> 10
<211> 4
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:amino acids
     used to link S3 multimer to remainder of
     polypeptide
<400> 10
Ile Glu Gly Arg
<210> 11
<211> 7
<212> PRT
<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence:enterokinase
      recognition sequence, enterokinase cleavage site
<220>
<221> MOD_RES
<222> (1)..(7)
<223> Xaa = any amino acid
```

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<220>
<221> MOD_RES
<222> (4)..(5)
<223> Asp at positions 4 and 5 may be present or absent
<400> 11
Xaa Asp Asp Asp Asp Lys Xaa
1 5
```

- P - V